(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 227 736 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
07.01.2004 Bulletin 2004/02

(21) Application number: **00977850.7**

(22) Date of filing: **20.10.2000**

(51) Int Cl.[7]: **A23J 3/00**, A23J 3/34, A23L 1/325, A23L 3/00, A61K 38/00

(86) International application number:
PCT/IS2000/000012

(87) International publication number:
WO 2001/028353 (26.04.2001 Gazette 2001/17)

(54) PROTEIN HYDROLYSATES PRODUCED WITH THE USE OF MARINE PROTEASES

PROTEINHYDROLYSATE HERGESTELLT UNTER VERWENDUNG VON MARINEN PROTEASEN

HYDROLYSATS PROTEIQUES OBTENUS A PARTIR DE PROTEASES MARINES

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
Designated Extension States:
LT LV

(30) Priority: **20.10.1999 IS 522599**

(43) Date of publication of application:
07.08.2002 Bulletin 2002/32

(73) Proprietor: **Nordur EHF**
104 Reykjavik (IS)

(72) Inventors:
- **BJARNASON, Jon, Bragi**
  IS-101 Reykjavik (IS)
- **BENEDIKTSSON, Bergur**
  IS-104 Reykjavik (IS)

(74) Representative: **Plougmann & Vingtoft A/S**
Sundkrogsgade 9
P.O. Box 831
2100 Copenhagen O (DK)

(56) References cited:
EP-A- 0 797 928
CA-A- 1 313 835
US-A- 5 532 007
WO-A-89/10960
US-A- 3 852 479

- **DATABASE WPI Section Ch, Week 199649 Derwent Publications Ltd., London, GB; Class B04, AN 1996-495762 XP002901515 & RU 2 055 482 C (AS USSR FAR E PACIFIC BIOORG CHEM INST), 10 March 1996 (1996-03-10)**

EP 1 227 736 B1

Description

TECHNICAL FIELD

[0001] The present invention relates to a method of hydrolyzing proteins, protein hydrolysates obtained by the method, and food products and non-food products comprising the hydrolysates of the invention.

BACKGROUND AND PRIOR ART

[0002] Methods of hydrolyzing proteins are well known in the art. Conventionally, protein hydrolysates are produced chemically by hydrolyzing protein or proteinaceous material, such as for example defatted soy flour or wheat gluten, with hydrochloric acid under refluxing conditions. The resulting hydrolysates are inexpensive and can have satisfactory organoleptic properties. However, chemical hydrolysis is accompanied by non-specific side reactions causing e.g. the formation of chlorohydrins, such as monochlorodihydroxypropanols (MCDPs) and dichloropropanols (DCPs), the presence of which is undesirable in food products. The food industry is demanding milder methods of modifying food products, reducing the need for harsh, chemical reaction conditions and for removal of side-reaction products and residual reagents.

[0003] Alternatively, protein or proteinaceous material may be hydrolyzed enzymatically. Typically, the relevant protein source is first subjected to a (partial) hydrolysis with one or more suitable endoproteases. Then, the resulting protein fragments can be degraded completely or partially into individual amino acids or dipeptides or tripeptides by the use of exopeptidases. Alternatively, the endopeptidases and exopeptidases may function simultaneously in an enzyme mixture resulting in a similar complete or partial degradation of the protein or proteinaceous material.

[0004] A fundamental problem in the enzymatic hydrolysis of proteins and proteinaceous material is the formation of a bitter flavor due to the formation of short peptide fragments. The bitter taste is believed to be the result of cleavage of proteins at amino acids with hydrophobic side chains, resulting in the formation of peptides with exposed hydrophobic side chains, which are typically inaccessible in proteins and longer peptides due to their tertiary structure.

[0005] In order to solve this problem, the state of the art proposes to use specific proteases to limit the degree of hydrolysis and obtain preferred terminal side-chains. For example, US 5,866,357 describes the use of a Glu/Asp specific protease for the preparation of hydrolysates, optionally with the use of an additional specific protease. Furthermore, WO 98/27827, for example, proposes, for the purpose of solving the problem, using a proteolytic enzyme mixture comprising only one exopeptidase, wherein the exopeptidase is produced using rDNA techniques, and may be employed in combination with one or more suitable endopeptidases, such as Fromase TM (Gist-Brocades, France) and Maxatase TM (Genencor International Belgium). US 5,532,007 discloses the use of a combination of purified enzymes, a neutral protease producible by means of a Bacillus strain and an alkaline protease producible by means of a Bacillus strain. The reference states that raw meat is preferably treated with proteases exclusively with endo-activities for the purpose of the described invention to obtain meat hydrolysates. Methods disclosed in WO 94/25580 employ a proteolytic enzyme preparation derived from the fungus Aspergillus oryzae (Flavorzyme™) comprising a mixture of endopeptidases and exopeptidases.

[0006] Alternatively, EP 0 823 998 A2, proposes the enzymatic hydrolysis of smoked meat as the protein or proteinaceous material for the generation of a protein hydrolysate which does not taste bitter. In this process it is preferred that only one enzyme, preferably a neutral or an alkaline protease having an endopeptidase effect, for example Pescalase (Gist Brocades), Alcalase (Novo Nordisk) or Promod 31 (Biocatalysts), be employed for the hydrolysis.

[0007] WO 89/10960 proposes the use of complex enzyme mixtures from krill to modificate protein, peptide and/or lipid constituents of biological material in industrial processes. The enzyme compositions disclosed are prepared by incubating macerated whole Antarctic krill (*Euphasia superba*) at 50°C for 20 h. Examples of various applications are described, such as hydrolysis of fish and meat. The krill enzyme preparations are said to contain different proteases and lipolytic enzymes, e.g. a considerable amount of phospholipases. Such preparations do not seem to have been used on a commercial scale for the production of food-grade hydrolysates. It should be noted that in all of these prior art references, hydrolysates are obtained by enzymatic incubation at 50-65°C.

[0008] WPI abstract AN 1996-495762 (RU-C-2 055 482) describes the use of pylloritic enzymes from Salmon for the production of protein-nucleic hydrolysates, but low-temperature hydrolysis is not discosed or anticipated. US 3,852,479 describes a process for producing protein hydrolysates having a high glutamic acid content, by hydrolyzing protein material with a glutaminase together with a proteolytic enzyme. Also herein are temperature above 50°C preferred. CA 1313835 describes the use of porcine trypsin or Enzeco AP-1™ protease (alkaline phosphatase of bacterial origin) for enzymatic extraxtion of carotenoprotein from crustacean wastes. None of these references disclose the use of proteases from Gadidae fish species for producing protein hydrolysates or suggest that such or other similar enzyme compositions would be effective at low temperatures.

[0009] For the production of many delicate processed food products originating from or simulating natural products,

such as, e.g. seafood soups and sauces, flavor extracts need not only have non-bitter properties but must also exhibit the same or a very similar flavor as the characterizing flavor of the natural product. Most quality-restaurants still prepare extracts for e.g. shellfish soups using conventional methods of making a stock from shells, claws, heads etc. Enzymatically prepared flavor agents have generally not been used widely as they do not seem to provide a satisfactory natural flavor.

[0010] The odors of fish and other seafood species are produced by complex mixtures of volatile compounds, and are very sensitive to conditions affecting the freshness of the species. Species-related odor compounds in fresh fish are present in very low levels, but many of such compounds have low odor thresholds and therefore, even though present in low levels (ppb) they still affect the overall aroma of the fish spedes and changes in their concentrations drastically affect the overall aroma. These compounds include unsaturated carbonyl compounds and alcohols with six, eight, or nine carbon atoms. Also, bromophenols in low concentrations have been associated with the natural, sea-, iodine, marine-like flavors of seafood. Microbial compounds are microbially formed during spoilage of fish. These include short chain alcohols, ketones, aldehydes, amines, sulphur compounds, aromatics and acids, and result mainly from the degradation of amino- and fatty acids. Proteolytic activity will accelerate the spoilage as small peptides and free amino acids are nutrients for bacteria, resulting in the formation of bad smelling metabolites. Therefore, careful selection of conditions for the hydrolysis of seafood and related protein materials is highly critical for the production of such hydrolysates for high-quality food products. with desirable organoleptic properties. It is likely that prolonged incubations at temperatures in the range of 50 - 65°C will degrade the overall flavor and aromatic character of the protein-containing materials being hydrolyzed, in particular of fish and other seafood material, due to the loss of volatile compounds and production of undesired side-reaction products.

[0011] There is a need for methods of hydrolyzing proteins under mild conditions leading to high yields and to hydrolysates with excellent organoleptic properties, in particular protein hydrolysates that have preserved the natural flavor of the protein-containing starting material such as seafood, but which do not have the bitter flavor which arises during conventional hydrolysis of protein-containing materials, It has been found that methods for obtaining hydrolysates at low enzyme incubation temperatures result in that the freshness and flavor (volatile flavor agents) of the raw material can be preserved, assumingly due to a reduced level of side-reactions and microbial activity and that a relatively non-specific enzyme preparation derived from fish intestines and obtainable with economical and technically simple methods can be used for effective hydrolysis of proteinaceous materials to obtain hydrolysates that are non-bitter and retain excellent flavor characteristics of the protein-containing raw material. A particularly advantageous aspect of the invention is the low temperature range at which such enzyme preparations are proteolytically active. It is conceivable that the low temperature range at which the methods and processes of the invention are preferably carried out at is an important factor contributing to the organoleptic properties of the obtained products.

SUMMARY OF THE INVENTION

[0012] Accordingly, in a first aspect, the invention provides a method for producing a protein hydrolysate from a natural protein-containing raw material, the method comprising the steps of: *a*) preparing an aqueous slurry comprising 1 - 100 %wt of protein-containing material; *b*) incubating the slurry with a proteolytic composition derived from a Gadidae species; *c*) agitating the slurry for 0.25 to 48 hours at a temperature in the range of 0 to 60°C; *d*) optionally inactivating the proteolytic mixture; and e) optionally separating the solution fraction from solid material.

[0013] In a further aspect, a protein hydrolysate is provided which is obtainable by the method of the invention.

[0014] In yet a further aspect, the invention further provides a food product comprising a protein hydrolysate obtainable according to the invention.

[0015] The invention provides in a still further aspect, a process for preparing a food product comprising the steps of obtaining a protein hydrolysate according to the invention and formulating a food product using the hydrolysate.

[0016] In another aspect of the invention, a process is provided for producing a flavor preparation, the process comprising the steps of a) preparing an aqueous slurry comprising 1 - 100 %wt protein-containing material; *b*) incubating the slurry with a proteolytic composition derived from a Gadidae species; c) agitating the slurry for 0,25 to 48 hours at a temperature in the range of 2 to 40°C; *d*) optionally inactivating the proteolytic mixture; *e*) separating the solution fraction from solid material; and *f*) concentrating the solution to a dry weight content of from 10 wt% to 98 wt%.

[0017] In still a further aspect of the invention, a non-food product is provided comprising a protein hydrolysate according to the invention.

[0018] In yet another aspect, the invention provides a method for releasing at least part of the astaxanthin from an astaxanthin-containing shellfish material, the method comprising the steps of preparing as the starting material an aqueous slurry comprising the shellfish material; incubating the slurry with a proteolytic composition derived from a Gadidae species, agitating the slurry at a temperature in the range of 2 to 60°C; and inactivating the proteolytic mixture to obtain a protein hydrolysate containing, relative to the starting material, a higher content of released astaxanthin.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The proteolytic compositions used according to the invention are derived from the intestines of Gadidae species (cold water fish) thus rendering them unusual characteristics such as, in particular, a high activity at low temperatures. The detailed characterization of a proteolytic preparation from Cod (*Gadus Morhua*) intestines has shown that the preparation comprises several proteolytic components. It has been observed that the preparation comprises at least five or more proteolytic enzyme components or groups. These proteolytic components or groups comprise the endopeptidases trypsin, at least three isozymes (Ásgeirsson et al., 1989), chymotrypsin, at least two isozymes (Ásgeirsson and Bjamason, 1991), and elastase, at least one isozyme (Ásgeirson and Bjarnason, 1993), and the exopeptidases of the aminopeptidase and carboxypeptidase types.

[0020] It has been found that such preparations not only serve as a useful source of the above individual enzyme components, but also surprisingly that they can be efficiently used for producing protein hydrolysates with unusual characteristics, i.e. which are non-bitter and retain the flavor characteristics of the protein-containing raw material.

[0021] As mentioned, the invention provides a method for producing a protein hydrolysate from a natural protein-containing raw material. The method comprises as a first step the preparation of an aqueous slurry comprising 1 to 100 wt% of protein-containing material, preferably 10 - 100 wt%, such as e.g. in the range of 25 - 100 wt%. The material may be pre-treated with conventional means such as mechanical shredding, cutting, mincing, or grinding. The content of protein-containing material in the slurry will depend on the water content and texture of the material, e.g. when hydrolyzing flesh of white fish with a dry weight content of 18 - 25 %, additional water may not be needed. However, for hydrolyzing material with a higher dry weight content, water is added to the slurry to obtain a dry weight content of the slurry of 1 - 30%, preferably 5 - 25%, such as 10 - 20%.

[0022] The slurry is thereafter incubated with a proteolytic preparation derived from a Gadidae species, and the slurry is agitated for 0.25 to 48 hours, preferably 1 to 10 hours, more preferably 1 to 6 hours, including 2 to 4 hours. The incubation is performed at any convenient temperature at which the enzyme composition does not become heat inactivated, i.e. in the range of from 0 °C to about 60, but preferably from 2 to about 40°C, including 5 to 35°C, and more preferably in the range of 5 to 30°C, such as 10 to 25°C, including 15°C, 17°C, and 20°C. In certain embodiments, a particularly low temperature range may be desirable, such as e.g. 2 -10 °C, including 2 - 8°C. Generally, lower incubation temperatures are compensated with longer incubation times or a higher relative concentration of the proteolytic composition, or both. Low incubation temperatures, such as temperatures below 40°C, particularly below 25-30°C are conceivably an important factor contributing to the retention of the flavor and aroma of fresh, delicate protein-containing materials to be used for food-grade hydrolysates, as discussed above.

[0023] In accordance with established practice, the proteases in the incubation mixture may optionally be suitably inactivated, such as e.g. by quick heating to above 60°C, such as to 70°C, or by decreasing the pH of the incubation mixture to a pH at which the proteases become inactivated, such as to below pH 5, as can be seen in Fig. 2 and Fig. 3 for a proteolytic preparation derived from Cod.

[0024] The solution fraction, containing dissolved, hydrolyzed peptides is then optionally separated from solid material, e.g. by sedimentation, filtration, or centrifugation. The enzyme inactivation may optionally be performed after such separation, on the solution fraction alone or both fractions separately.

[0025] For certain protein-containing materials, it is advantageous to heat the material prior to the step of hydrolysis, to minimize eventual microbial contamination, and/or to inactivate other enzymes causing undesired reactions. The inventors have found that e.g. when hydrolyzing lobster flesh, quick heating to a temperature of 70 - 90°C eliminates the activity of phenyloxidases that otherwise will darken the lobster flesh and the resulting hydrolysate.

[0026] As a further optional step, the solution fraction may be concentrated to a desired dry matter content by any applicable means such as evaporative heating under vacuum or lyophilization. The dry matter content of the hydrolysate product is anywhere in the range of 1 to 100%, depending on the desired form and liquid content of the product. liquid or semi-liquid hydrolysate preparations will typically have a dry matter content of 5 - 40%, including 15 - 35%. The hydrolysate product may alternatively be in the form of a gel or a paste, or in a solid or semi-solid form such as e.g. flakes, a powder form, or as compressed bodies, e.g. as tablets, bars, cubes, or blocks.

[0027] The incubation is performed at a pH in the range of 6 to 11, preferably between 7 to 10. As demonstrated with a Cod-derived proteolytic composition in Fig. 1 and Fig. 2, the method of the invention performs reasonably well even at the rather extreme conditions, i.e. at pH values in the full range of 6 to 11.

[0028] The proteolytic composition is derived from, *Gadidae* species (cod species and hakes). Atlantic Cod (*Gadus morhua*) is a readily available and particularly useful source of the composition. Other fish species which are used as a source of the proteolytic composition in further embodiments include haddock, and pollack (saithe). Preferred species include in particular those from which the intestines can readily be separated from other viceral organs.

[0029] In a particularly useful embodiment, the proteolytic composition is provided by a process comprising the steps of mixing water with fish viscera; agitating the mixture for a period of 0.5 hours or longer, such as in the range of 1 - 10 hours, and preferably 2 - 6 hours; separating solid residue from the solution, e.g. by sedimentation, filtration, or

centrifugation; and concentrating the aqueous solution to obtain the proteolytic composition. Fish viscera in this context comprise fish intestines, e.g. the pyloric caeca, however, in a preferred embodiment, the liver, milt, roe and the stomach are removed from the viscera prior to the preparation. The inventors have observed that most of the preferred active proteases are derived from the less acidic intestines and not the more acidic stomach. The concentration step is performed with any suitable means well known to the person skilled in the art, such as, e.g. ultrafiltration, to obtain a desired activity per volume unit, such as, e.g. in the range of 0.1 - 100 BAPNA units/mL measured as described in Example 1, more preferably in a range of 0.5 - 10 BAPNA U/mL, including 1 - 5 BAPNA U/mL.

[0030] The agitation of the water-viscera mixture is performed at a pH and temperature at which the stability of the desired proteases is not decreased substantially from their optimum, such as e.g. at a temperature in the range of 0 - 20°C, and at a pH in the range of 6 - 11. In a preferred embodiment, the agitation of the water-viscera mixture is performed at a pH in the range of 6 to 9, including a range of 6 to 8, such as e.g. 6 to 8, and preferably at a temperature in the range of 0 to 15°C, including 0 - 10°C, more preferably 0 - 5°C.

[0031] The proteolytic composition comprises in one embodiment of the invention, at least one enzyme selected from the group consisting of trypsin, chymotrypsin, elastase, collagenase, aminopeptidase, and a carboxypeptidase type enzyme. Preferably, the composition comprises two or more of the above-mentioned enzymes, such as e.g. all of the enzymes. However, in certain useful embodiments, the proteolytic composition is further refined by the removal of at least one of its enzyme components. Example 2 demonstrates how elastase and serine collagenases are removed from a proteolytic composition derived from Cod to obtain a modified Cod-derived enzyme composition with many of the same characteristics as the basic composition of Example 1.

[0032] The proteolytic composition used according to the invention, preferably has an enzymatic activity in the range of 0,1 to 100 BAPNA units/mL, measured as in the assay of Example 1 herein, more preferably 0.5 to 20 BAPNA units/mL, even more preferred 1 - 10 units/mL, including 1 to 5 units/mL. The suitable amount of enzyme composition to incubate the protein material slurry with will depend on the specific activity of the enzyme composition, the desired time-range for the hydrolysis incubation, and the particular type of protein raw material used. In one embodiment the slurry is incubated with the proteolytic composition at an amount of 1 - 10.000 BAPNA proteolytic units/kg of protein-containing raw material, preferably 10 - 500 units/kg, including 25 - 250 units/kg, such as e.g. 25 - 100 units/kg.

[0033] In yet another embodiment, at least one non-fish enzyme preparation is added to the proteolytic composition derived from a Gadidae species prior to the incubation with protein-containing raw material. Such non-fish enzyme preparations include neutral or alkaline proteases such as the commercial protease preparations Neutrase™, Alcalase™ and Flavorzyme™ (from Novo Nordisk, Denmark), Pescalase™ and Fromase™ (both from Gist-Brocades, France), Maxatase™ (Genencor International, Belgium), and Promod 31™ (Biocatalysts, UK); lipase preparations such as fungal lipases, e.g. the commercial lipase preparations Palatase™M and Palatase™A (both from Novo Nordisk, Denmark); glycolytic preparations wherein the glycolytic preparation comprises at least one amylase, glucanase, glutaminase, phytase, glycosidase, cellulase, chitinase or pectinase; and any combinations of the above.

[0034] In a further useful embodiment of the invention, the aqueous slurry is treated with at least one enzyme preparation such as e.g. one of the above-mentioned preparations, prior to and/or after treatment with the proteolytic composition derived from a Gadidae species.

[0035] The protein-containing material which is hydrolyzed with the method according to the invention is in one embodiment an animal protein selected from the group consisting of a fish protein, shellfish protein, milk protein, whey protein, casein, meat protein, blood protein, egg protein, elastin and gelatin. In another embodiment the protein-containing material is a vegetable protein selected from the group consisting of soy protein; grain protein, e.g. wheat gluten or zein; rapeseed protein; alfalfa protein, pea protein, fabaceous bean protein; cottonseed protein; and sesame seed protein.

[0036] In a particularly useful embodiment of the invention, the protein-containing material is marine organism material, such as whole fish, fish flesh, fish viscera, fish skin, fish bones or any part or mixture thereof; or a marine organism material derived from one or more crustacean or a mollusc species including shrimps, lobsters, crayfish, crabs, clams, oysters, and mussels, wherein the material comprises-whole animals, flesh, shells or any parts, mixtures or combinations thereof.

[0037] In a yet further useful embodiment, the protein-containing raw material comprises proteinaceous membrane or skin, such as from fish, fish liver, swim bladder of fish, inner body cavity of fish, fish eggs or roe.

[0038] Another useful embodiment uses animal-derived protein-containing raw material such as from lamb, pork, beef, chicken, and turkey, wherein the material is either raw or cooked such as by boiling, frying, or curing, and comprises muscle tissue, tendons, other connective tissue, bones, offal or any part or mixture thereof.

[0039] It has been found that the method of the invention may be used in combination with a fermentation process, i.e. wherein the protein-containing material is hydrolyzed under fermentation conditions to obtain a fermented protein hydrolysate, and/or wherein the protein hydrolysate obtained by a method according to the invention is subjected to a fermentation process. Examples of such fermentation processes are processes fermenting fish (e.g. in production of fish sauce) cocoa beans or soy (such as in production of soy sauce, tempeh, miso). The addition of the proteolytic

enzyme preparation or addition of protein hydrolysate may be useful for decreasing the total process time of the fermentation such as in the curing of herring.

[0040] In another aspect, the invention provides protein hydrolysates obtained by the method of the invention. In useful embodiments the protein hydrolysates are derived from animal protein such as any of the above-mentioned. Other embodiments include protein hydrolysates derived from a vegetable protein such as all those previously mentioned.

[0041] In a further aspect of the invention, a food product is provided, which product comprises a protein hydrolysate according to the invention. The amount of protein hydrolysate incorporated in the food product will typically be in the range of 0.1-50% by weight, such as 0.1 - 5 wt%.

[0042] In a particularly useful embodiment of the invention, the food product is a flavor preparation for use in a food product such as, e.g. a soup, a sauce, a broth, a paté, a mousse, a soufflé, a cheese, frying dough, orly dough, and pastries.

[0043] Another food product of the present invention is an ingredient of a mother milk substitute for infants. Due to the high degree of hydrolysis obtained by the method of the invention, the protein hydrolysate of the invention may advantageously be incorporated in mother milk substitutes, the hydrolysate having a significantly lower allergenicity than non-hydrolyzed milk proteins.

[0044] Yet another food product of the invention includes the protein hydrolysate of the invention as a protein supplement or to provide other properties of the food product. Thus, the protein hydrolysate incorporated in the food product may for instance be based on fish or fish offal, or meat or scrap meat, rendered from bone by subjecting crushed bone to the method of the invention. Other proteinaceous by-products from the fish industry may also be used by the method of the present invention to generate protein hydrolysates of the invention as a protein supplement or to provide other properties of the food product.

[0045] A further aspect of the invention provides a process for preparing a food product, comprising the steps of obtaining a protein hydrolysate according to the invention, and formulating a food product using the hydrolysate. Food products suitably prepared with the process of the invention include all of the above-mentioned.

[0046] In another aspect, the invention provides a process of producing a flavor preparation, which process comprises the steps of: preparing an aqueous slurry comprising 1 - 100 wt% of protein-containing material, such as e.g. 25 to 100 wt% protein-containing material, depending on the water content and texture of the material; incubating the slurry with a proteolytic composition derived from a Gadidae species; agitating the slurry for 0,25 to 48 hours, such as in the range of 1 to 10 hours, preferably 1.5 to 6 hours, at a temperature in the range of 2 to 40°C; optionally inactivating the proteolytic mixture; separating the solution fraction from solid material; and concentrating the solution to a dry weight content of from 10 wt% to 98 wt%.

[0047] The optional inactivation step is suitably performed as described above, similarly the agitation step and the concentrating step may be performed with any suitable methods such as those above-mentioned.

[0048] In a preferred embodiment, the temperature during the proteolytic incubation is in the range of 5 - 30°C, such as in a range of 10 - 20°C or a range of 10 - 30°C, including 15°C to 25°C. The incubation may be performed at any pH at which the proteolytic composition used is active, in one embodiment the pH during the incubation is in the range of 6 to 11, preferably from 6 to 9.

[0049] In a useful embodiment, the invention provides a process for a seafood flavor preparation, wherein the protein-containing material is derived from seafood or seafood byproducts, such as material derived from fish species including cod, haddock, saithe, halibut, flounder, eel, monkfish, salmon, trout, and ocean perch, herring, capelin, and other seafood species including sea urchin, shrimp, lobster, crayfish, crabs, clams, oysters, and mussels.

[0050] In a further embodiment, the process according to the invention is for producing a meat flavor preparation, wherein the protein-containing material is derived from meat or meat byproducts. In advantageous embodiments the protein-containing material is derived from one or more species from beef, lamb, pork, reindeer, and poultry species including chicken, turkey, ducks, and ostrich.

[0051] In a still further aspect, the invention provides a non-food product comprising a protein hydrolysate according to the invention. The flavor characteristics obtained by the method of the invention are advantageous for production of feed products and pet food.

[0052] Furthermore, generation of highly hydrolyzed protein hydrolysates from fish gelatine may improve gelatine products for incorporation into cosmetics, such as creams and shampoos.

[0053] The use of the protein hydrolysate of the invention as fermentation medium mentioned above is further makes the hydrolysates of the invention useful for other fermentations as well, such as for a fermentation broth, in particular in the field of medicine for the production of pharmaceutical.

[0054] In yet another aspect, the invention provides a method for releasing at least part of the astaxanthin from an astaxanthin-containing shellfish material, the method comprising the steps of preparing as the starting material an aqueous slurry as described comprising the shellfish material; incubating the slurry with a proteolytic composition derived from a Gadidae species such as described above, agitating the slurry at a temperature in the range of 2 to

60°C; and inactivating the proteolytic mixture to obtain a protein hydrolysate containing, relative to the starting material, a higher content of released astaxanthin.

[0055] As a further optional step, the method comprises the separation of an astaxanthin-containing aqueous phase, by any suitable means such as sedimentation, filtration, or centrifugation.

[0056] The following examples further illustrate the present invention.

EXAMPLE 1: Preparation of a mixture of proteases from cod

[0057] 100 kg of frozen cod viscera, without liver milt and roe were, thawed and added to a four-fold volume of cold potable water in an extraction tank and the pH adjusted to pH 8 to 9 with a sodium hydroxide solution. The mixture was stirred for 2-6 hours at 0 to 5°C. After a brief period of crude sedimentation (30 minutes) the aqueous extract was run off the remaining insoluble viscera with a pump and collected in a sedimentation tank. The aqueous extract was allowed to stand in the cooled sedimentation tank to sediment for 24 to 60 hours. The supernatant was decanted from the supernatant tank to a holding tank using a pump. The supernatant was concentrated 10 to 20-fold by ultrafiltration and diafiltered to an acceptable level of ionic strength with conductivity below about 3 mS/cm. 10-15 litres of ultrafiltratered and diafiltered protease preparation was obtained and referred to as Cryotin. The protease preparation had a proteolytic activity of 1.5 BAPNA units/mL. Repeated preparations using the described procedure gave batch preparations with an activity between 1 to 5 BAPNA units/mL.

[0058] The activity is measured with an assay using the synthetic substrate Benzyl-Arg-p-nitroanilide (BAPNA) at a final substrate concentration of 1 mM in 50 mM Tris-HCl, 10 mM CaCl, pH 8.1, at 25°C. The absorbance increase is measured at 405 nm and the BAPNA activity calculated according to a molar absorbtivity constant of 8800 $M^{-1}cm^{-1}$.

EXAMPLE 2: Purification of cod elastase and cod serine collagenases from concentrated cod viscera extract

[0059] 10-15 litres of ultrafiltratered and diafiltered concentrate as obtained in Example 1 was applied to a couple of 1 litre packed chromatography columns connected in a series, the first containing a CM fast flow cation exchange resin (Pharmacia, Sweden), and the second one a DEAE fast flow anion exchange resin (Pharmacia, Sweden) The columns were pre-equilibrated with 10 column volumes of 25 mM Tris buffer of pH 7.8, containing 2.5 mM calcium chloride (buffer A). The concentrate was pumped onto the columns at a flow rate of 100 ml per minute. When the application of the concentrated solution onto the columns was completed, residual material was washed off the continuous column system with 8 L of buffer A. The flow through fraction containing trypsin, chymotrypsin and the exopeptidases is collected and termed Cryotin X.

After this wash was completed, the columns were individually eluted with 5 column volumes of a high salt solution of 25 mM Tris buffer pH 7.8 containing 0.5 M NaCl and 2.5 mM calcium chloride. The cod elastase was thus desorbed from the CM column and the serine collagenases from the DEAE column.

EXAMPLE 3: Hydrolysis and release of protein and proteinaceous material from shrimp shells using Cryotin.

[0060] Shrimp shells and water were mixed together in the ratio of 1:0.75 (w/w), by adding 3000 g of shrimp shells to 2250 g of water. An aliquot of 130 ml of Cryotin enzyme mixture of Example 1 containing 1.4 U/ml (BAPNA units), i.e. a total of 182 units per the 3000 g of shrimp shells, or 0.06 BAPNA hydrolyzing units per gram of shrimp shells. This mixture was reacted by stirring it in a rotating drum for 5 hours at a speed of 40 cycles per minute at room temperature (20° C). The solution fraction was separated from the solid residue by filtration through a course sieve followed by cheesecloth filtration. During the course of the 5-hour reaction-time samples were taken from the reaction mixture for monitoring the reaction. For comparative purposes the reaction was repeated with the same materials except 130 ml of water was added instead of the Cryotin enzyme mixture, and samples were taken for measurements.

[0061] With an assay for soluble amino acids and peptides, using TCA (trichloroacetic acid) precipitation followed by reaction with Folin Ciocalteu's reagent, it was revealed that the Cryotin reaction generated more than six fold the amount of soluble amino acids and peptides in five hours compared to the batch with no Cryotin. Furthermore, the Cryotin containing batch generated soluble amino acids and peptides approximately linearly with time throughout the five hour reaction period while the Cryotin-free batch released a burst of soluble amino acids and peptides only in the first hour of the reaction, indicative of the release of already soluble amino acids and peptides but no hydrolysis of protein.

An assay for astaxanthin, employing measurements of absorbance at 468 nm, was used to monitor its release from the shrimp shells. These measurements showed more than a 2.5 fold release of the pigment with the enzymatic Cryotin method than the Cryotin-free method, which probably released pigment primarily with mechanical sheer forces.

EXAMPLE 4: Comparison of hydrolysis and solubilization of protein and proteinaceous material from shrimp shells using Cryotin and Alcalase 2.4L.

[0062] Shrimp shells and water were mixed together in the ratio of 1:0.75 (w/w), by adding 6000 g of shrimp shells to 4500 g of water. An aliquot of 266 ml of Cryotin enzyme preparation of Example 1 containing 110 U/ml (Azocoll units), i.e. a total of 29247 Azocoll units per the 6000 g of shrimp shells, or about 4.87 Azocoll hydrolyzing units per gram of shrimp shells. The mixture was reacted by stirring it in a rotating drum for 5 hours at a speed of 40 cycles per minute at room temperature (20° C). The solution fraction was separated from the solid residue by filtration through a course sieve followed by cheesecloth filtration. During the course of the 5-hour reaction-time samples were taken from the reaction mixture for monitoring the reaction.

[0063] For comparative purposes the reaction was repeated with the same materials except now the shrimp shell water mixture was reacted with Alcalase 2.4L. Shrimp shells and water were mixed together in the ratio of 1:0.75 (w/w), by adding 2500 g of shrimp shells to 1875 g of water. An aliquot of 0.25 ml of the Alcalase 2.4L enzyme mixture containing 46794.6 U/ml (Azocoll units), i.e. a total of 11699 Azocoll units per the 2500 g of shrimp shells, or about 4.68 Azocoll hydrolysing units per gram of shrimp shells. The mixture was reacted by stirring it in a rotating drum for 5 hours at a speed of 40 cycles per minute at room temperature (about 20° C). The solution fraction was separated from the solid residue by filtration through a course sieve followed by cheesecloth filtration. During the course of the 5-hour reaction time samples were taken from the reaction mixture for monitoring the reaction.

[0064] The Azocoll assay was used to measure comparable activity of Alcalase and Cryotin of Example 1, as Alcalase is known to have very low activity towards BAPNA or GPR. The activity is measured in the following manner: Samples of 50 mg of Azocoll are measured into small glass tubes, 2 samples of Azocoll per each concentration of the enzyme sample to be assayed. Phoshate buffer (100 mM, pH 8.0) was added to each tube to a total final volume of 5 mls. Enzyme samples to be assayed were added to the tubes after the tubes were allowed to stand with only buffer and Azocoll for 5 to 10 minutes. After addition of enzyme samples to the assay mixture the system was allowed to incubate at room temperature for 15 minutes after which the absorbance at 520 nm was measured. The results were first observed as a linear relationship between absorbance at 520 nm vs. amount of enzyme, from which the number of Azocoll hydrolysing units per ml enzyme were calculated, where 1 Azocoll unit (AU) is the amount of enzyme giving rise to an increase in absorbance of 0.1 units.

[0065] With an assay for soluble amino acids and peptides, using TCA (trichloroacetic acid) precipitation followed by reaction with Folin Ciocalteu's reagent, it was revealed that the Cryotin reaction generated more than eight-fold the amount of soluble amino acids and peptides in four hours as compared to the batch with Alcalase. An assay for astaxanthin, employing measurements of absorbance at 468 nm, was used to monitor its release from the shrimp shells. These measurements showed more than a 1.6 fold release of the pigment with the enzymatic Cryotin method compared to the Alcalase reaction.

EXAMPLE 5: Production of food flavourants from shrimp shells using Cryotin.

[0066] Shrimp shells (20% dry matter) and water were mixed together in a ratio of 1:0.63 (w/w), by adding 3980 g of shrimp shells to 2525 g of water. An aliquot of 165 ml of Cryotin enzyme mixture of Example 1 containing 1.4 U/ml (BAPNA units), i.e. a total of 231 units per the 3980 g of shrimp shells, or 0.058 BAPNA hydrolyzing units per gram of shrimp shells. This mixture was reacted by stirring it in a rotating drum for 4 hours at a speed of 40 cycles per minute at a temperature of 15° C. The solution fraction was separated from the solid residue by filtration through a course sieve followed by cheese cloth filtration. The solid residue was used for chitin production. The aqueous fraction was heated to 70° C for 10 minutes to inactivate the enzymes and pasteurize the fluid. The aqueous phase was then concentrated to 30% dry matter content by evaporation at 45° C under vacuum.

EXAMPLE 6: Hydrolysis and release of proteinaceous material as feed from shrimp shells using Cryotin

[0067] Shrimp shells and water was mixed together in the ratio of 1:0.5 (w/w), by adding 3000 g of shrimp shells to 1500 g of water. An aliquot of 130 ml of Cryotin enzyme mixture of Example 1 containing 1.4 U/ml (BAPNA units), i.e. a total of 182 units per the 3000 g of shrimp shells, or about 0.06 BAPNA hydrolyzing units per gram of shrimp shells. This mixture was reacted by stirring it in a rotating drum for 10 hours at a speed of 40 cycles per minute at a temperature of 30° C. The solution fraction was separated from the solid residue by filtration through a course sieve followed by cheese cloth filtration. The solid residue was used for chitin production. The aqueous fraction was heated to 70° C for 10 minutes to inactivate the enzymes and pasteurize the fluid. The aqueous phase was then concentrated to 40% dry matter content by evaporation at 45° C under vacuum, after which the concentrate was dehydrated further in a drier.

EXAMPLE 7: A flavor preparation from whole shrimp

[0068] Frozen whole shrimp (22.2% dry matter) was minced in a grinder. The ground shrimp and water was mixed together in a ratio of 1:0.75 (w/w), by adding 6700 g of ground shrimp to 5010 g of water. An aliquot of 311 ml of Cryotin enzyme preparation of Example 1 containing 1.4 U/ml (BAPNA units), i.e. a total of 435.4 units to the 6700 g of ground whole shrimp, or 0.065 BAPNA hydrolyzing units per gram of ground whole shrimp. This mixture was reacted by stirring it in a rotating drum for 4 hours at a speed of 40 cycles per minute at a temperature of 17°C. The solution fraction was separated from the solid residue by filtration through a course sieve followed by cheese cloth filtration and sedimentation for 10 to 20 minutes. The solid residue was used for chitin production. The aqueous fraction was heated to 70°C for 10 minutes to inactivate the enzymes and pasteurize the fluid. The aqueous phase was then concentrated to 30 to 40% dry matter content by evaporation at 45°C under vacuum.

[0069] The experiment was repeated with the same ratio of raw materials, water and enzyme preparation, except the incubation temperature was kept at 20°C and the evaporative drying performed at 50°C, resulting in a product of essentially similar quality.

EXAMPLE 8: A flavor preparation from lobster

[0070] Lobster heads and claws or whole lobster is used in this preparation, and the raw material is either fresh or frozen. Frozen whole lobster was minced in a grinder. The ground lobster and water was mixed together in a ratio of 1:0.75 (w/w), by adding 8540 g of ground lobster to 6410 g of water. The mixture of lobster and water was brought to 90°C for a short time (to inactivate, in particular, fenyloxidase present in the lobster) and then cooled down to process temperature which in this example was 35° C. An aliquot of 385 ml of the Cryotin mixture of Example 1 containing 1.4 U/ml (BAPNA units), i.e. a total of 539 units to the 8540 g of ground whole lobster, or about 0.063 BAPNA hydrolysing units per gram of ground whole lobster. This mixture was reacted by stirring it in a rotating drum for 1.5 hours at a speed of 40 cycles per minute at a temperature of 35° C. The solution extract was separated from the solid residue by filtration through a course sieve followed by cheese cloth filtration and sedimentation for 10 to 20 minutes. The solid residue was used for chitin preparation. The aqueous fraction was heated to 70° C for 10 minutes to inactivate the enzymes and pasteurize the fluid. The aqueous phase was then concentrated to 40 to 50% dry matter content by evaporation at 45°C under vacuum.

Subsequent experiments were performed with same ratio of raw materials, water and enzyme preparation, except the pre-heating temperature was 70°C, and the incubation temperature was kept at 30°C, resulting in resulting in a product of essentially similar quality. Same results were achieved with the final concentration step performed at 50°C to obtain a product of 30 to 40% dry matter content.

EXAMPLE 9: Flavor preparation from pollack

[0071] As raw material it is possible to use either the Pollack flesh, i.e. skinned filets of Pollack or Pollack bones, and the raw material may be either fresh or frozen. Fresh skinned Pollack filets were minced in a grinder. The ground Pollack filets and water were mixed together by adding 4750 g of ground Pollack filets with a dry weight content of 21 % to 4685 g of water. An aliquot of 292 ml of the Cryotin mixture of Example 1 containing 1.4 U/ml (BAPNA units), i. e. a total of 409 units to the 4750 g of ground Pollack filets or 0.086 BAPNA hydrolyzing units per gram of ground Pollack filets. This mixture was reacted by stirring it in a rotating drum for 4 hours at a speed of 40 cycles per minute at a temperature of 26°C. The solution extract fraction was separated from the solid residue by filtration through a course sieve followed by cheesecloth. The aqueous fraction was heated to 70° C for 10 minutes to inactivate the enzymes and pasteurize the fluid. The aqueous phase was then concentrated to 25 to 35% dry weight content by evaporation at 45°C under vacuum.

The experiment was repeated with the same ratio of raw materials, water and enzyme preparation, except the incubation temperature was kept at 30°C and the evaporative drying performed to obtain a product of 18 - 25% dry weight, resulting in a product of essentially similar quality.

EXAMPLE 10: Preparation of shrimp soup using flavor preparation from whole shrimp

[0072] The following soup recipe is an example of application of the extracts of food flavorants for human consumption. In soup making the extract can ether be used as base material as in the following recipe or as top material, that is added in small amount to almost prepared soup to give it the final taste.

Shrimp soup for 12 people

Ingredients:

[0073]

2 onions
2 carrots
2.5 litres water (or fish stock)
2 tablespoons fish bullion
1 tablespoon curry powder
250 g shrimp extract of Example 7
1 garlic clove, chopped
100 g butter, melted + 100 g flour
200 mL cream
100 mL white wine

[0074] The onions and carrots are chopped and stir fried in a saucepan. To the saucepan are added the 2.5 litres water (or fish stock), 2 tablespoons fish bullion, 1 tablespoon curry, 250 g shrimp extract of Example 7, 1 garlic clove, chopped. Mixed together and simmered for 20 minutes, then the soup stock is strained. The soup stock is thickened with a mixture of 100 g butter, melted and 100 g flour added to the melted butter. The cream is stirred in the soup and the soup is reheated. Then the white wine is added and whole shrimps for decoration. The soup is ready for serving.

EXAMPLE 11: Removal of membrane from squid by using a proteolytic preparation from Cod.

[0075] Two pieces of frozen whole squid, 300 g each, were placed in a rotating drum together with 450 mL water and 300 mL of Cod-derived proteolytic composition produced as in Example 1, the pH of which had been adjusted to 8.2. The proteolytic composition had an activity of 105 GPR units/mL, such that total proteolytic activity was 31400 GPA units, or 52 GPR units/g of squid (1.7 BAPNA units/g). The pH was adjusted to 8.0 several times during the incubation. The temperature was kept at 10°C or below, and the squid was inspected and samples taken after 15, 30, 60, 150, 210, and 330 minutes.

[0076] The activity is measured with assay using the synthetic substrate Gly-L-Pro-L-Arg-p-nitroanilide (GPR or sGPRpna) at a substrate concentration of 0.3 mM in 50 mM Tris-HCl, 10 mM CaCl, pH 8.1, at 25°C. The absorbance increase is measured at 410 nm and the GPR activity calculated according to a molar absorbtivity constant of 8800 M^{-1} cm^{-1}. GPR units are converted to BAPNA units according to the equation $U_{BAPNA} = U_{GPR} / 31$.

[0077] The following table lists the observations and measurements performed during the course of the experiment.

Table 11.1

time, min.	temp., °C	pH	A_{280}	comments
0	2	8.1	0.105	Squid pieces frozen
15	2	6.9	0.270	Loosening of membrane observed, one piece becoming slimy on surface, pH adjusted w/ NaOH.
30	3	7.8	0.368	Unchanged from last inspection, pH adjusted w/ NaOH.
60	4	7.6	0.510	Membrane has started to disintegrate. Bladders full of liquid appear on surface. Membrane more slimy. pH adjusted w/ NaOH.
150	8	7.4	0.527	Membrane is loosening shape. pH adjusted w/ NaOH.
210	10	7.6	0.580	Dark part of membrane has almost disappeared from body, still remaining on tail. pH adjusted.
330	10	-	0.690	Membrane has almost disappeared from the body and tail.

[0078] It is observed that a proteolytic composition efficiently removes proteinaceous skin and membrane from adhering tissue, such as the membrane on the surface of squid. A corresponding experiment was performed disintegrating the membrane sack of Cod roe.

EXAMPLE 12: Preparation of hydrolysates from lamb meat

[0079] 107 g fried ground lamb meat was mixed with 163 mL water. The temperature of the slurry was about 25°C. 7 mL of Cryotin with an activity of about 1.4 BAPNA units/ mL were mixed with the slurry (i.e. about 0.09 U/g of meat) and the pH adjusted to 7.3 with the addition of NaOH. The slurry was stirred for 4 h. at 20°C and sample aliquots removed at 1-hour intervals for analysis (pH measurement and absorbance at 280 nm). After completion of the 4 hour incubation, the hydrolysate had a characteristic flavor and aroma of lamb meat. Table 12.1 shows the absorbance measurements for the sampled aliquots, indicating the release of soluble peptides and amino acids.

Table 12.1

time, hours	pH	A_{280}
0	7.32	0.014
1	6.98	0.083
2	6.86	0.102
3	6.85	0.130
4	6.85	0.146

[0080] The experiment was repeated with raw ground lamb meat and similar results were obtained.

EXAMPLE 13: Sensory testing of shrimp and lobster hydrolysates

[0081] A sample of shrimp flavor preparation according to example 7 was subjected to a sensory testing by a test panel consisting of 6 panelists. Before testing, the preparation was diluted by adding 20 g of taste preparation to 200 mL water and heated to 50°C. The sample was served in small plastic cups. The panelists first evaluated the sample individually by judging the aroma, color, and taste and noted descriptive features and subsequently discussed the general sensory characteristics of the test sample.

[0082] Table 13.1 lists comments made by individual panelists.

Table 13.1

color	aroma	taste
pink (salmon)	sweet, faint aroma, haddock smell	sweet taste, shrimpflesh, haddock, fishflesh

[0083] The panel subsequently reached the following joint conclusion: The shrimp taste preparation had a very characteristic taste and smell of shrimp, esp. a taste of shrimpflesh (also taste of fishflesh).

EXAMPLE 14: Hydrolysis of protein-containing materials with a refined proteolytic composition without elastase and serine collagenases.

[0084] All experiments of Examples 3 to 13 are repeated, except that the Cod-derived proteolytic composition of Example 2 (termed Cryotin X) is used as the hydrolyzing agent. Essentially the same ratio of proteolytic activity, measured as in Example 1, is used, to obtain corresponding results as those described in Examples 3 to 13.

[0085] **Figure 1:** The stability of the Cod-derived proteolytic composition of Example 1 was measured as a function of temperature and time by observing residual enzyme activity after 24 hour incubations at pH values of 6, 8 and 10 and temperatures of 10°, 30° and 45° C. From this figure one can observe that the Proteolytic composition has similar stability characteristics at the three pH values, but is much more stable at low temperatures than high. Enzyme activity was monitored by absorbance measurement at 247 nm at using tosyl arginine methyl ester (TAME). The activity is determined at 25°C using TAME at a final concentration of 0.075 mM, the assay buffer consists of 40 mM Tris/HCl pH 8.1 and 10 mM $CaCl_2$. One unit of activity is calculated at 1 μmol substrate hydrolyzed/min using a molar extinction coefficient of 540 $M^{-1}cm^{-1}$ at 247 nm.

[0086] **Figure 2:** The activity of the Cod-derived proteolytic composition of Example 1 is shown as a function of pH using TAME as a substrate. The buffers used at a final concentration of 0.1 M were Hepes/HCl (pH 5.4 to 8.0) and glycinate (pH 8.3 to 10.8) containing 10 mM calcium chloride. From this figure one can observe that the composition has relatively good TAME hydrolyzing activity through the pH range between 7 and 10.8, with a maximum between pH about 8.0 and 10.5, where the activity is determined as described in the legend of Figure 1.

[0087] **Figure 3:** The temperature dependence of the activity of the Cod-derived proteolytic composition of Example 1. The enzyme activity was monitored using tosyl arginine methyl ester (TAME) as substrate at a final concentration of 0.75 mM. The assay buffer consisted of 40 mM Tris/HCl (pH 8.1) and 10 mM calcium chloride and activity was monitored by following absorbance at 247 nm. From this figure one can observe increasing hydrolytic activity of the composition up to a maximum at around 50°C, after which the activity decreases rapidly due to temperature denaturation. (Activity determined as in legend of Figure 1.)

Claims

1. A method for producing a protein hydrolysate from a natural protein-containing raw material, the method comprising the steps of:

   a) preparing an aqueous slurry comprising 1 - 100 % wet weight of protein-containing material,
   b) incubating the slurry with a proteolytic composition derived from a *Gadidae* species,
   c) agitating the slurry for 0.25 to 48 hours at a temperature in the range of 0 to 60°C,
   d) optionally inactivating the proteolytic mixture, and
   e) optionally separating the solution fraction from solid material.

2. The method according to claim 1 wherein the proteolytic composition is derived from fish selected from the group consisting of cod, haddock, and pollack.

3. The method according to claim 1 wherein the proteolytic composition is derived from cod.

4. The method according to claim 1, wherein the proteolytic composition is provided by a process comprising the steps of:

   i) mixing waterwith fish viscera
   ii) agitating the mixture for a period of 0.5 hours or longer
   iii) separating solid. residue from solution
   iv) concentrating the aqueous solution to obtain the proteolytic composition.

5. The method according to claim 4, wherein the agitation is performed at a temperature in the range of 0 to 10°C, and at a pH in the range of 6 to 9.

6. The method according to claim 1, wherein the proteolytic composition comprises at least one enzyme selected from the group consisting of trypsin, chymotrypsin, elastase, collagenase, aminopeptidase, and a carboxypeptidase type enzyme.

7. The method according to claim 1, wherein the proteolytic composition has a proteolytic activity in the range of 0,1 to 50 BAPNA units/mL, measured as in the assay of Example 1 herein.

8. The method according to claim 6, wherein the proteolytic composition has a proteolytic activity in the range of 0,5 to 10 BAPNA units/mL.

9. The method according to claim 1, wherein the slurry is incubated with the proteolytic composition at an amount of 10 - 1000 BAPNA proteolytic units/kg of protein-containing raw material, preferably 25 - 250 BAPNA proteolytic units/kg.

10. The method according to claim 1, wherein the proteolytic composition is further refined by the removal of at least one of its enzyme components.

11. The method according to claim 1, wherein at least one non-fish enzyme preparation is added to the proteolytic composition derived from a *Gadidae* species.

12. The method according to claim 1, wherein the aqueous slurry is treated with at least one enzyme preparation prior to or after treatment with the proteolytic composition derived from a *Gadidae* species.

13. The method according to claim 11 or 12, wherein the at least one enzyme preparation is a lipase preparation.

14. The method according to claim 11 or 12, wherein the at least one enzyme preparation comprises one or more enzyme components selected from the group consisting of_amylase, glucanase, glutaminase, phytase, glycosidase, cellulase, chitinase and pectinase.

15. The method according to claim 1, wherein step c) is performed at a temperature in the range of 2 to 40°C.

16. The method according to claim 1, wherein step c) is performed at a temperature in the range of 5 to 30°C.

17. The method according to claim 1, wherein step c) is performed at a pH of between 6 and 11, preferably between 6 and 9.

18. The method according to claim 1, wherein the protein-containing material is an animal protein selected from the group consisting of a fish protein, shellfish protein, milk protein, whey protein, casein, meat protein, blood protein, egg protein, elastin and gelatin.

19. The method according to claim 1, wherein the protein-containing material is a vegetable protein selected from the group consisting of soy protein; grain protein, e.g. wheat gluten or zein; rapeseed protein; alfalfa protein, pea protein, fabaceous bean protein; cottonseed protein; and sesame seed protein.

20. The method according to claim 1, wherein the protein-containing material is marine organism material.

21. The method according to claim 20, wherein the marine organism material is a material selected from the group consisting of whole fish, fish flesh, fish offal, fish viscera, fish skin, fish bones and any part or mixture thereof.

22. The method according to claim 20, wherein the protein-containing material is a material including whole animals, flesh, shells or any parts, mixtures or combinations thereof derived from a marine organism selected from the group consisting of crustaceans and molluscs.

23. The method according to claim 22, wherein the crustacean or mollusc is selected from the group consisting of shrimps, lobsters, crayfish, crabs, clams, oysters, and mussels.

24. The method according to claim 18, wherein the protein-containing raw material comprises proteinaceous membrane or skin, such as from fish, fish liver, swim bladder of fish, inner body cavity of fish, fish eggs or roe.

25. The method according to claim 18, wherein the protein-containing raw material is selected from the group comprising lamb, pork, beef, chicken, and turkey.

26. The method according to claim 25, wherein the protein-containing material is raw or cooked and comprises muscle tissue, tendons, other connective tissue, bones, offal, and any part or mixture thereof.

27. The method according to any of the claims 1-26, wherein the protein-containing material is hydrolysed under fermentation conditions to obtain a fermented protein hydrolysate.

28. The method according to any of the claims 1-26 comprising as a further step that the protein hydrolysate is subjected to a fermentation process.

29. A protein hydrolysate obtainable by the method of any of the preceding claims.

30. The protein hydrolysate according to claim 29, wherein the hydrolyzed protein is derived from an animal protein selected from the group consisting of fish protein, shell fish protein, milk protein, whey protein, casein, meat protein, blood protein egg protein, elastin and gelatin.

31. The protein hydrolysate according to claim 29, wherein the hydrolyzed protein is derived from a vegetable protein selected from the group consisting of soy protein; grain protein such as wheat gluten or zein; rapeseed protein; alfalfa protein, pea protein, fabaceous bean protein; cotton seed protein; and sesame seed protein.

32. A food product comprising a protein hydrolysate according to any of claims 29-31.

33. The food product according to claim 32 which is a flavor preparation for use in a product selected from the group consisting of a soup, a sauce, a broth, a paté, a mousse, a soufflé, a cheese, frying dough, orly dough, and pastries.

34. A process for preparing a food product comprising the steps of (i) obtaining a protein hydrolysate according to any of claims 29-31 and (ii) formulating a food product using the hydrolysate.

35. A process of producing a flavor preparation, the process comprising the steps of

a) preparing an aqueous slurry comprising 1 -100 % wet weight protein-containing material,
b) incubating the slurry with a proteolytic composition derived from a Gadidae species,
c) agitating the slurry for 0,25 to 48 hours at a temperature in the range of 2 to 40°C,
d) optionally inactivating the proteolytic mixture,
e) separating the solution fraction from solid material,
f) concentrating the solution to a dry weight content of from 10 wt% to 98 wt%.

36. The process according to claim 35, wherein the flavor preparation is a seafood flavor preparation, and the protein-containing material is derived from seafood or seafood byproducts.

37. The process according to claim 35, wherein the flavor preparation is a meat flavor preparation, and the protein-containing material is derived from meat or meat byproducts.

38. The process according to claim 35, wherein the incubation in step c) is performed at a temperature in the range of 5 to 30°C.

39. The process according to claim 35, wherein the incubation in step c) is performed at a pH in the range of from 6 to 9.

40. The process according to claim 35, wherein the proteolytic composition is obtained by the method of claim 4.

41. The process according to claim 36, wherein the protein-containing material is derived from one or more species selected from the group containing cod, haddock, saithe, halibut, flounder, eel, monkfish, salmon, trout, ocean perch, herring, capelin, sea urchin, shrimp, lobster, crayfish, crabs, clams, oysters, and mussels.

42. The process according to claim 37, wherein the protein-containing material is derived from one or more species selected from the group containing beef, lamb, pork, reindeer, and poultry species including chicken, turkey, duck, and ostrich.

43. A non-food product comprising a protein hydrolysate according to any of claims 29-31.

44. The non-food product according to claim 43 which is a product selected from the group consisting of a feed product, a pet food, a cosmetic, a fermentatlon broth, and a pharmaceutical product.

45. A method for releasing at least part of the astaxanthin from an astaxanthin-containing shellfish materlal, the method comprising the steps of preparing as the starting material an aqueous slurry comprising the shellfish material; incubating the slurry with a proteolytic composition derived from a Gadidae species, agitating the slurry at a temperature in the range of 2 to 60°C; and inactivating the proteolytic mixture to obtain a protein hydrolysate containing, relative to the starting material, a higher content of released astaxanthin.

46. A method according to claim 45 comprising as a further step the separation of an astaxanthin-containing aqueous phase.

Patentansprüche

1. Verfahren zum Herstellen eines Proteinhydrolysats aus einem natürlichen, proteinhaltigen Rohmaterial, wobei das Verfahren die folgenden Schritte umfasst:

a) Herstellen einer wässrigen Schlämme umfassend 1 - 100 % des Nassgewichtes an proteinhaltigem Material,

b) Inkubieren der Schlämme mit einer proteolytischen Zusammensetzung, die von einer *Gadidae*-Art abgeleitet ist,

c) Rühren der Schlämme für 0,25 bis 48 Stunden bei einer Temperatur im Bereich von 0 bis 60 °C,

d) optional Inaktivieren der proteolytischen Mischung und

e) optional Trennen des Lösungsanteils von festem Material.

2. Verfahren nach Anspruch 1, wobei die proteolytische Zusammensetzung von Fisch ausgewählt aus der Gruppe bestehend aus Kabeljau, Schellfisch und Seelachs abgeleitet ist.

3. Verfahren nach Anspruch 1, wobei die proteolytische Zusammensetzung von Kabeljau abgeleitet ist.

4. Verfahren nach Anspruch 1, wobei die proteolytische Zusammensetzung durch ein Verfahren bereit gestellt ist, das die folgenden Schritte umfasst:

i) Mischen von Wasser mit Fischeingeweiden

ii) Rühren der Mischung über einen Zeitraum von 0,5 Stunden oder länger

iii) Trennen des festen Rückstands von der Lösung

iv) Konzentrieren der wässrigen Lösung, um die proteolytische Zusammensetzung zu erhalten.

5. Verfahren nach Anspruch 4, wobei das Rühren bei einer Temperatur im Bereich von 0 bis 10 °C und bei einem pH-Wert im Bereich von 6 bis 9 durchgeführt wird.

6. Verfahren nach Anspruch 1, wobei die proteolytische Zusammensetzung mindestens ein Enzym ausgewählt aus der Gruppe bestehend aus Trypsin, Chymotrypsin, Elastase, Collagenase, Aminopeptidase und einem Enzym vom Carboxypeptidasetyp umfasst.

7. Verfahren nach Anspruch 1, wobei die proteolytische Zusammensetzung eine proteolytische Aktivität im Bereich von 0,1 bis 50 BAPNA Einheiten/ml, wie in dem Test von Beispiel 1 hierin gemessen, besitzt.

8. Verfahren nach Anspruch 6, wobei die proteolytische Zusammensetzung eine proteolytische Aktivität im Bereich von 0,5 bis 10 BAPNA Einheiten/ml besitzt.

9. Verfahren nach Anspruch 1, wobei die Schlämme mit der proteolytischen Zusammensetzung in einer Menge von 10 - 1000 BAPNA proteolytischen Einheiten/kg proteinhaltigem Material, bevorzugt 25 - 250 BAPNA proteolytischen Einheiten /kg, inkubiert wird.

10. Verfahren nach Anspruch 1, wobei die proteolytische Zusammensetzung durch die Entfernung mindestens eines ihrer Enzymbestandteile weiter verfeinert wird.

11. Verfahren nach Anspruch 1, wobei mindestens eine nicht aus Fisch stammende Enzymzubereitung der proteolytischen Zusammensetzung, die von einer *Gadidae*-Art abgeleitet ist, hinzugefügt wird.

12. Verfahren nach Anspruch 1, wobei die wässrige Schlämme vor oder nach der Behandlung mit der proteolytischen Zusammensetzung, die von einer *Gadidae*-Art abgeleitet ist, mit mindestens einer Enzymzubereitung behandelt wird.

13. Verfahren nach Anspruch 11 oder 12, wobei die mindestens eine Enzymzubereitung eine Lipasezubereitung ist.

14. Verfahren nach Anspruch 11 oder 12, wobei die mindestens eine Enzymzubereitung einen Enzymbestandteil oder mehrere Enzymbestandteile ausgewählt aus der Gruppe bestehend aus Amylase, Glukanase, Glutaminase, Phy-

tase, Glykosidase, Cellulase, Chitinase und Pektinase umfasst.

15. Verfahren nach Anspruch 1, wobei Schritt c) bei einer Temperatur im Bereich von 2 bis 40 °C durchgeführt wird.

16. Verfahren nach Anspruch 1, wobei Schritt c) bei einer Temperatur im Bereich von 5 bis 30 °C durchgeführt wird.

17. Verfahren nach Anspruch 1, wobei Schritt c) bei einem pH-Wert zwischen 6 und 11, vorzugsweise zwischen 6 und 9 durchgeführt wird.

18. Verfahren nach Anspruch 1, wobei das proteinhaltige Material ein tierisches Protein ausgewählt aus der Gruppe bestehend aus einem Fischprotein, Schalentierprotein, Milchprotein, Molkeprotein, Casein, Fleischprotein, Blutprotein, Eiprotein, Elastin und Gelatine ist.

19. Verfahren nach Anspruch 1, wobei das proteinhaltige Material ein pflanzliches Protein ausgewählt aus der Gruppe bestehend aus Sojaprotein; Getreideprotein, z.B. Weizengluten oder Zein; Rapssamenprotein; Alfalfaprotein, Erbsenprotein, Bohnenprotein; Baumwollsamenprotein und Sesamsamenprotein ist.

20. Verfahren nach Anspruch 1, wobei das proteinhaltige Material ein Material aus einem im Meer lebenden Organismus ist.

21. Verfahren nach Anspruch 20, wobei das Material aus einem im Meer lebenden Organismus ein Material ausgewählt aus der Gruppe bestehend aus ganzem Fisch, Fischfleisch, Fischabfall, Fischeingeweiden, Fischhaut, Fischgräten/Fischknochen und einem beliebigen Teil oder einer beliebigen Mischung davon ist.

22. Verfahren nach Anspruch 20, wobei das proteinhaltige Material ein Material ist, das ganze Tiere, Fleisch, Gehäuse oder beliebige Teile, Mischungen oder Kombinationen davon umfasst, die von einem im Meer lebenden Organismus, ausgewählt aus der Gruppe bestehend aus Schalentieren und Weichtieren, abgeleitet sind.

23. Verfahren nach Anspruch 22, wobei das Schalentier oder das Weichtier ausgewählt ist aus der Gruppe bestehend aus Garnelen, Hummern, Flusskrebsen, Krabben, Venusmuscheln, Austern und Miesmuscheln.

24. Verfahren nach Anspruch 18, wobei das proteinhaltige Rohmaterial proteinöse Membran oder Haut, wie beispielsweise von Fischen, Fischleber, der Schwimmblase von Fischen, der inneren Körperhöhle von Fischen, Fischeiern oder Rogen, umfasst.

25. Verfahren nach Anspruch 18, wobei das proteinhaltige Rohmaterial ausgewählt ist aus der Gruppe bestehend aus Lamm, Schwein, Rind, Hühnchen und Truthahn.

26. Verfahren nach Anspruch 25, wobei das proteinhaltige Rohmaterial roh oder gekocht ist und Muskelgewebe, Sehnen, anderes Bindegewebe, Knochen, Abfall und einen beliebigen Teil bzw. eine beliebige Mischung davon umfasst.

27. Verfahren nach einem der Ansprüche 1-26, wobei das proteinhaltige Material unter Fermentationsbedingungen hydrolysiert wird, um ein fermentiertes Proteinhydrolysat zu erhalten.

28. Verfahren nach einem der Ansprüche 1-26, das als einen weiteren Schritt umfasst, dass das Proteinhydrolysat einem Fermentationsprozess unterzogen wird.

29. Proteinhydrolysat, das durch das Verfahren eines der vorherigen Ansprüche erhalten werden kann.

30. Proteinhydrolysat nach Anspruch 29, wobei das hydrolysierte Protein von einem tierischen Protein ausgewählt aus der Gruppe bestehend aus Fischprotein, Schalentierprotein, Milchprotein, Molkeprotein, Casein, Fleischprotein, Blutprotein, Eiprotein, Elastin und Gelatine abgeleitet ist.

31. Proteinhydrolysat nach Anspruch 29, wobei das hydrolysierte Protein von einem pflanzlichen Protein bestehend aus Sojaprotein; Getreideprotein, wie z.B. Weizengluten oder Zein; Rapssamenprotein; Alfalfaprotein, Erbsenprotein, Bohnenprotein; Baumwollsamenprotein und Sesamsamenprotein abgeleitet ist.

32. Nahrungsmittelprodukt umfassend ein Proteinhydrolysat nach einem der Ansprüche 29-31.

33. Nahrungsmittelprodukt nach Anspruch 32, das eine Geschmackszubereitung zur Verwendung in einem Produkt ausgewählt aus der Gruppe bestehend aus einer Suppe, einer Sauce, einer Brühe, einer Pastete, einer Mousse, einem Soufflé, einem Käse, einem Frittierteig, einem Orly-Teig und Gebäck ist.

34. Verfahren zum Herstellen eines Nahrungsmittelproduktes umfassend die Schritte des (i) Erhaltens eines Proteinhydrolysats nach einem der Ansprüche 29-31 und des (ii) Formulierens eines Nahrungsmittelproduktes unter Verwendung des Hydrolysats.

35. Verfahren zum Herstellen einer Geschmackszubereitung, wobei das Verfahren die folgenden Schritte umfasst:

a) Herstellen einer wässrigen Schlämme umfassend 1 - 100 % des Nassgewichts an proteinhaltigem Material,

b) Inkubieren der Schlämme mit einer proteolytischen Zusammensetzung, die von einer Gadidae-Art abgeleitet ist,

c) Rühren der Schlämme für 0,25 bis 48 Stunden bei einer Temperatur im Bereich von 2 bis 40°C,

d) optional Inaktivieren der proteolytischen Mischung,

e) Trennen des Lösungsanteils von dem festen Material,

f) Konzentrieren der Lösung auf einen Trockengewichtgehalt zwischen 10 Gew.-% und 98 Gew.-%.

36. Verfahren nach Anspruch 35, wobei die Geschmackszubereitung eine Meeresfrüchtegeschmackszubereitung ist und das proteinhaltige Material von Meeresfrüchten oder Meeresfrüchte-Nebenprodukten abgeleitet ist.

37. Verfahren nach Anspruch 35, wobei die Geschmackszubereitung eine Fleischgeschmackszubereitung ist und das proteinhaltige Material von Fleisch oder Fleisch-Nebenprodukten abgeleitet ist.

38. Verfahren nach Anspruch 35, wobei die Inkubation in Schritt c) bei einer Temperatur im Bereich von 5 bis 30 °C durchgeführt wird.

39. Verfahren nach Anspruch 35, wobei die Inkubation in Schritt c) bei einem pH-Wert im Bereich von 6 bis 9 durchgeführt wird.

40. Verfahren nach Anspruch 35, wobei die proteolytische Zusammensetzung durch das Verfahren von Anspruch 4 erhalten wird.

41. Verfahren nach Anspruch 36, wobei das proteinhaltige Material von einer Art bzw. mehreren Arten ausgewählt aus der Gruppe bestehend aus Kabeljau, Schellfisch, Pollack, Heilbutt, Flunder, Aal, Seeteufel, Lachs, Forelle, Rotbarsch, Hering, Kapelan, Seeigel, Garnelen, Hummer, Langusten, Krabben, Venusmuscheln, Austern und Miesmuscheln abgeleitet ist.

42. Verfahren nach Anspruch 37, wobei das proteinhaltige Material von einer Art bzw. mehreren Arten ausgewählt aus der Gruppe bestehend aus Rind, Lamm, Schwein, Rentier und Geflügelarten einschließlich Hühnchen, Truthahn, Ente und Strauß, abgeleitet ist.

43. Nicht-Nahrungsmittelprodukt umfassend ein Proteinhydrolysat nach einem der Ansprüche 29-31.

44. Nicht-Nahrungsmittelprodukt nach Anspruch 43, das ein Produkt ausgewählt aus der Gruppe bestehend aus einem Futterprodukt, einem Haustierfutter, einem Kosmetikprodukt, einer Fermentationsbrühe und einem pharmazeutischen Produkt ist.

45. Verfahren zum Freisetzen mindestens eines Teils des Astaxanthins aus einem astaxanthinhaltigen Schalentiermaterial umfassend die Schritte des Herstellens einer wässrigen Schlämme als das Ausgangsmaterial umfassend das Schalentiermaterial; Inkubieren der Schlämme mit einer proteolytischen Zusammensetzung, die von einer

Gadidae-Art abgeleitet ist; Rühren der Schlämme bei einer Temperatur im Bereich von 2 bis 60 °C; und Inaktivieren der proteolytischen Mischung, um ein Proteinhydrolysat zu erhalten, das relativ zum Ausgangsmaterial einen höheren Gehalt an frei gesetztem Astaxanthin enthält.

46. Verfahren nach Anspruch 45, das als einen weiteren Schritt die Trennung einer astaxanthinhaltigen wässrigen Phase umfasst.

Revendications

1. Dispositif visant à produire un hydrolysat de protéine à partir d'une matière première naturelle contenant des protéines, comprenant les étapes suivantes :

a) préparation d'une suspension aqueuse comprenant 1 - 100 % (poids mouillé) de matière protéique,

b) incubation de la suspension avec une composition protéolytique provenant de l'espèce Gadidae,

c) agitation de la suspension pendant 15 minutes à 48 heures à une température comprise entre 0 et 60 °C,

d) (facultatif) inactivation du mélange protéolytique, et

e.) (facultatif) séparation de la fraction de solution de la matière solide.

2. Dispositif selon la revendication 1 dans lequel la composition protéolytique provient d'un poisson appartenant au groupe des cabillauds, églefins et lieux jaunes.

3. Dispositif selon la revendication 1 dans lequel la composition protéolytique provient du cabillaud.

4. Dispositif selon la revendication 1 dans lequel la composition protéolytique est réalisée selon un processus comprenant les étapes suivantes :

i) mélange de l'eau avec des viscères de poisson

ii) agitation du mélange pendant une demi heure ou plus

iii) séparation des résidus solides de la solution

iv) concentration de la suspension aqueuse afin d'obtenir la composition protéolytique.

5. Dispositif selon la revendication 4 dans lequel le mélange est agité à une température comprise entre 0 et 10 °C et avec un pH compris entre 6 et 9.

6. Dispositif selon la revendication 1 dans lequel la composition protéolytique comprend au moins une enzyme sélectionnée dans le groupe comprenant la trypsine, la chymotrypsine, l'elastase, la collagénase, l'aminopeptidase et une enzyme du type carboxypeptidase.

7. Dispositif selon la revendication 1 dans lequel la composition protéolytique possède une activité protéolytique comprise entre 0,1 à 50 unités de BAPNA/ml, mesurée selon le dosage de l'Exemple 1 décrit ici.

8. Dispositif selon la revendication 6 dans lequel la composition protéolytique possède une activité protéolytique comprise entre 0,5 à 10 unités de BAPNA/ml.

9. Dispositif selon la revendication 1 dans lequel la suspension est incubée avec la composition protéolytique à un taux de 10 - 1000 unités protéolytiques de BAPNA/kg de matière première protéique, et de préférence de 25 - 250 unités protéolytiques de BAPNA/kg.

10. Dispositif selon la revendication 1 dans lequel la composition protéolytique est ensuite affinée grâce au retrait d'au moins un de ses composants enzymatiques.

11. Dispositif selon la revendication 1 dans lequel au moins une préparation enzymatique, ne provenant pas de poisson, est ajoutée à la composition protéolytique provenant de l'espèce Gadidae.

12. Dispositif selon la revendication 1 dans lequel la suspension aqueuse est traitée avec au moins une préparation enzymatique avant ou après avoir été traitée avec la composition protéolytique provenant de l'espèce Gadidae.

13. Dispositif selon les revendications 11 ou 12 dans lequel ladite préparation enzymatique est une préparation de lipase.

14. Dispositif selon les revendications 11 ou 12 dans lequel ladite préparation enzymatique comprend un ou plusieurs composants enzymatiques sélectionné(s) dans le groupe comprenant l'amylase, la glucanase, la glutaminase, la phytase, la glucosidase, la cellulase, la chitinase et la pectinase.

15. Dispositif selon la revendication 1 dans lequel l'étape c) est effectuée à une température comprise entre 2 et 40 °C.

16. Dispositif selon la revendication 1 dans lequel l'étape c) est effectuée à une température comprise entre 5 et 30 °C.

17. Dispositif selon la revendication 1 dans lequel l'étape c) est effectuée avec un pH compris entre 6 et 11, et de préférence entre 6 et 9.

18. Dispositif selon la revendication 1 dans lequel la matière protéique est une protéine animale sélectionnée dans le groupe comprenant les protéines de poisson, les protéines de crustacés et mollusques, les protéines de lait, les protéines de lactosérum, la caséine, les protéines de viande, les protéines du sang, les protéines des oeufs, l'élastine et la gélatine.

19. Dispositif selon la revendication 1 dans lequel la matière protéique est une protéine végétale sélectionnée dans le groupe comprenant les protéines de soja, les protéines de céréales (comme par exemple le gluten du blé ou la zéine), les protéine de graines de colza, les protéines d'alfafa, les protéines de pois, les protéines de fabacées, les protéines de graines de coton et les protéines de graines de sésame.

20. Dispositif selon la revendication 1 dans lequel la matière protéique est un organisme marin.

21. Dispositif selon la revendication 20 dans lequel l'organisme marin est sélectionné parmi le poisson entier, la chair du poisson, les abats du poisson, les viscères du poisson, la peau du poisson, les arrêtes du poisson et n'importe quelle partie ou n'importe quel mélange de ces éléments.

22. Dispositif selon la revendication 20 dans lequel la matière protéique est une matière provenant des animaux entiers, de la chair, des coquilles ou n'importe quelle partie, combinaison ou n'importe quel mélange de ces éléments, provenant d'un organisme marin sélectionné parmi des crustacés et des mollusques.

23. Dispositif selon la revendication 22 dans lequel le crustacé ou le mollusque est sélectionné dans un groupe comprenant les crevettes, les homards, les écrevisses, les crabes, les palourdes, les huîtres et les moules.

24. Dispositif selon la revendication 18 dans lequel la matière première protéique comprend une membrane protéique ou une peau comme celle du poisson, du foie de poisson, de la vessie natatoire du poisson, de la cavité interne du corps du poisson, des oeufs ou de la rogue de poisson.

25. Dispositif selon la revendication 18 dans lequel la matière première protéique est sélectionnée dans un groupe comprenant l'agneau, le porc, le boeuf, le poulet et la dinde.

26. Dispositif selon la revendication 25 dans lequel la matière protéique est brute ou cuite et comprend du tissu musculaire, des tendons, d'autres tissus conjonctifs, des os, des abats, et n'importe quelle partie ou n'importe quel mélange de ces éléments.

27. Dispositif selon n'importe laquelle des revendications de 1 à 26 dans lequel la matière protéique est hydrolysée dans des conditions de fermentation afin d'obtenir un hydrolysat protéique fermenté.

28. Dispositif selon n'importe laquelle des revendications de 1 à 26 comprenant comme étape supplémentaire la sou-

mission de l'hydrolysat protéique à un processus de fermentation.

29. Hydrolysat protéique pouvant être obtenu grâce au dispositif de n'importe laquelle des revendications précédentes.

30. Hydrolysat protéique selon la revendication 29 dans lequel la protéine hydrolysée provient d'une protéine animale sélectionnée dans un groupe comprenant les protéines de poisson, les protéines de mollusques et de crustacés, les protéines de lait, les protéines de lactosérum, la caséine, les protéines de viande, les protéines du sang, les protéines des oeufs, l'élastine et la gélatine.

31. Hydrolysat protéique selon la revendication 29 dans lequel la protéine hydrolysée provient d'une protéine végétale sélectionnée dans le groupe comprenant les protéines de soja, les protéines de céréales (comme par exemple le gluten du blé ou la zéine), les protéine de graines de colza, les protéines d'alfafa, les protéines de pois, les protéines de fabacées, les protéines de graines de coton et les protéines de graines de sésame.

32. Produit alimentaire comprenant un hydrolysat protéique selon n'importe laquelle des revendications de 29 à 31.

33. Produit alimentaire selon la revendication 32 qui est une préparation aromatisante destinée à être utilisée dans un des produits suivants: soupe, sauce, bouillon, pâté, mousse, soufflé, fromage, pâte à frire, pâte « orly » et pâtisserie.

34. Méthode visant à préparer un produit alimentaire comprenant (i) l'obtention d'un hydrolysat protéique selon n'importe laquelle des revendications de 29 à 31 et (ii) la formulation d'un produit alimentaire utilisant l'hydrolysat.

35. Méthode visant à produire une préparation aromatisante et comprenant les étapes suivantes :

a) préparation d'une suspension aqueuse comprenant 1-100 % (poids mouillé) de matière première protéique,

b) incubation de la suspension avec une composition protéolytique provenant de l'espèce Gadidae,

c) agitation de la suspension pendant 15 minutes à 48 heures à une température comprise entre 2 et 40 °C,

d) (facultatif) inactivation du mélange protéolytique,

e) séparation de la fraction de solution de la matière solide,

f) concentration de la solution jusqu'à l'obtention d'un contenu d'un poids sec de 10 à 98 %.

36. Dispositif selon la revendication 35 dans lequel la préparation aromatisante est une préparation aromatisée aux fruits de mer, et la matière protéique provient de fruits de mer ou de sous-produits de fruits de mer.

37. Dispositif selon la revendication 35 dans lequel la préparation aromatisante est une préparation aromatisée à la viande, et la matière protéique provient de viande ou de sous-produits carnés.

38. Dispositif selon la revendication 35 dans lequel l'incubation de l'étape c) est effectuée à une température comprise entre 5 et 30 °C.

39. Dispositif selon la revendication 35 dans lequel l'incubation de l'étape c) est effectuée avec un pH compris entre 6 et 9.

40. Dispositif selon la revendication 35 dans lequel la composition protéolytique est obtenue selon le dispositif de la revendication 4.

41. Dispositif selon la revendication 36 dans lequel la matière protéique provient de l'une ou de plusieurs des espèces sélectionnées dans un groupe comprenant le cabillaud, l'églefin, le lieu noir, le flétan, le flet, l'anguille, la baudroie, le saumon, la truite, la perche de l'océan, le hareng, le capelan, l'oursin, la crevette, le homard, l'écrevisse, les crabes, les palourdes, les huîtres et les moules.

42. Dispositif selon la revendication 37 dans lequel la matière protéique provient de l'une ou de plusieurs des espèces

sélectionnées dans un groupe comprenant le boeuf, l'agneau, le porc, le chevreuil et les volailles telle que le poulet, la dinde, le canard et l'autruche.

43. Produit non alimentaire comprenant un hydrolysat protéique selon n'importe laquelle des revendications 29 à 31.

44. Produit non alimentaire selon la revendication 43 qui est un produit sélectionné dans un groupe comprenant les produits d'alimentation animale, les aliments pour animaux domestiques, les produits cosmétiques, les bouillons de fermentation et les produits pharmaceutiques.

45. Dispositif visant à libérer au moins une partie de l'astaxanthine présente dans un mollusque ou un crustacé contenant de l'astaxanthine, le dispositif comprenant les étapes suivantes : préparation d'une suspension aqueuse comprenant de la matière de mollusque ou crustacé comme matière de départ, incubation de la suspension avec une composition protéolytique provenant de l'espèce Gadidae, agitation de la suspension à une température comprise entre 2 et 60 °C, inactivation du mélange protéolytique afin d'obtenir un hydrolysat protéique ayant une quantité plus importante d'astaxanthine libérée par rapport à la matière de départ.

46. Dispositif selon la revendication 45 comprenant comme étape supplémentaire la séparation d'une phase aqueuse contenant de l'astaxanthine.

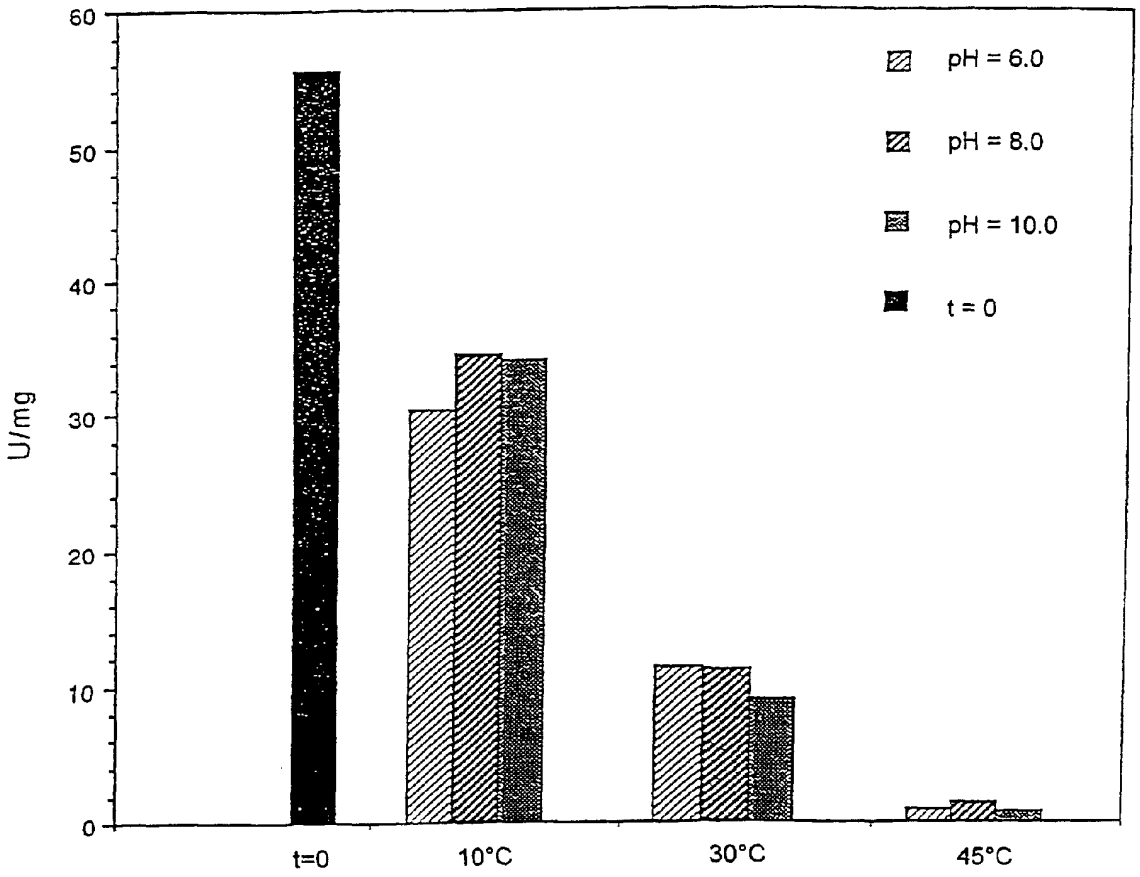

Fig. 1

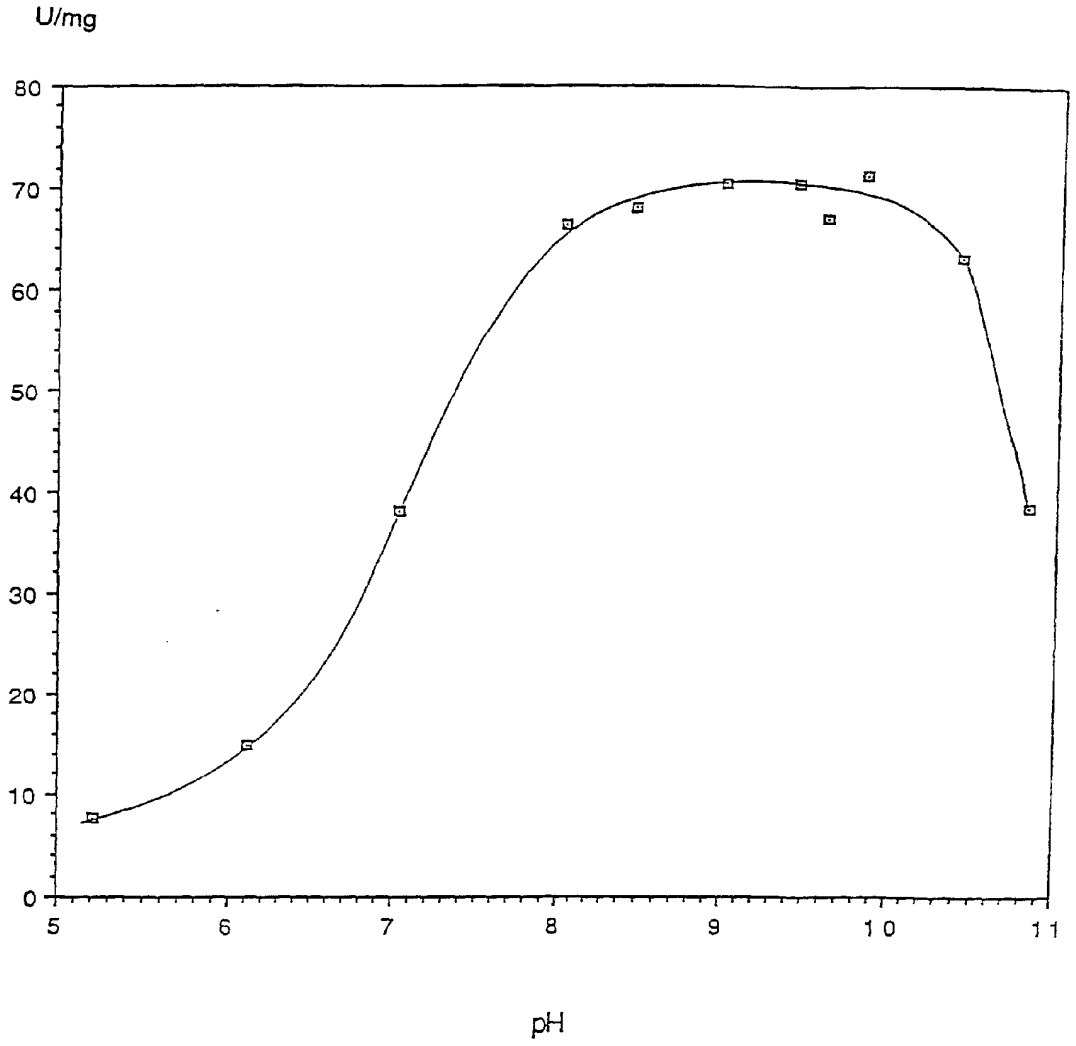

Fig. 2

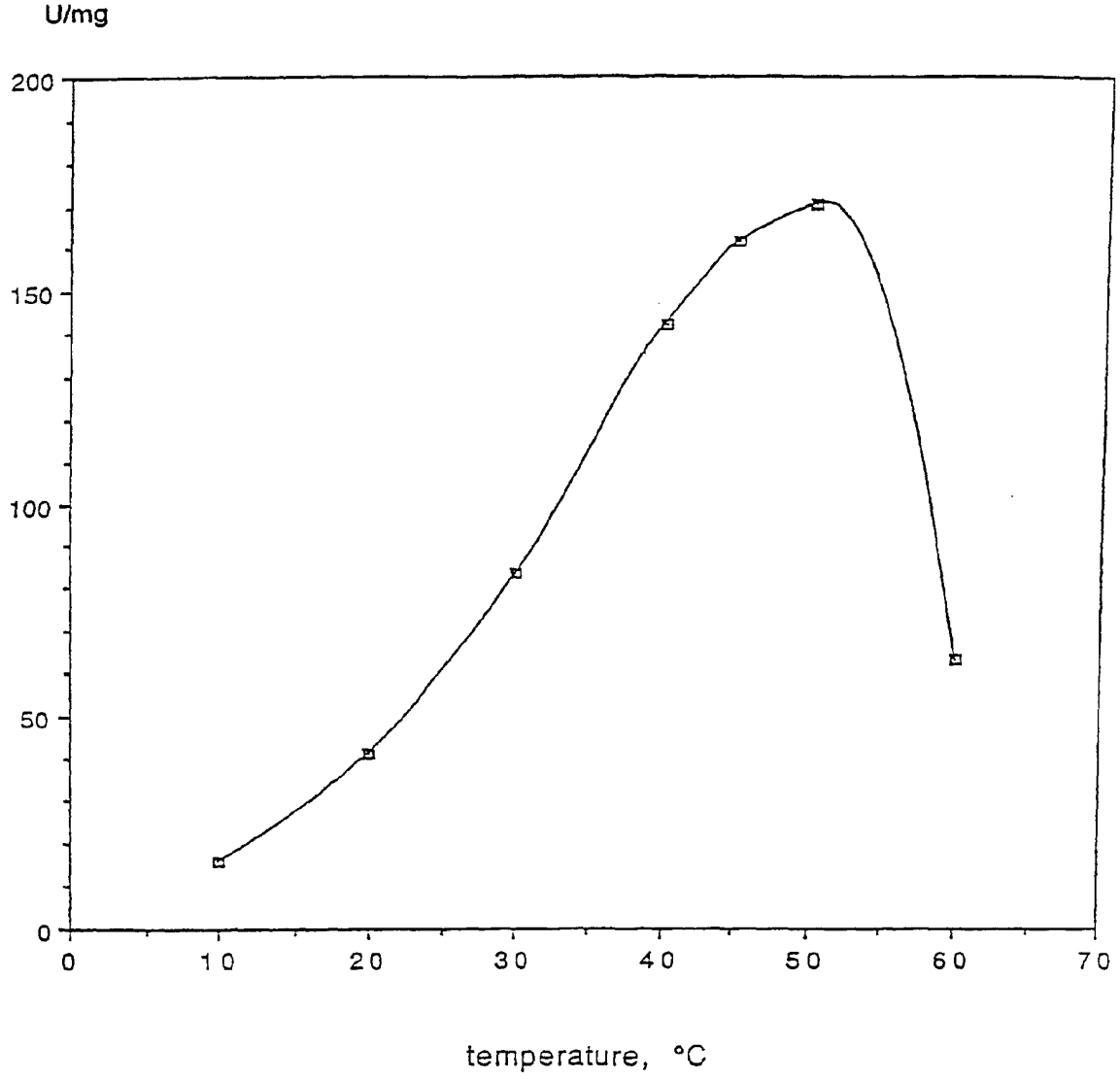

Fig. 3